(7) F. M. Allen, *J. Am. Med. Assocn.*, 89 (1927), 1577.
(8) C. B. Jordan, J. P. Whelan and W. F. Gidley, JOUR. A. PH. A., 13 (1924), 206.

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DETERMINATION OF OIL AND ALKALOIDS IN DELPHINIUM SEED.

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Previous researches on Delphinium seed, conducted by the writer,[1,2] made it desirable to determine the alkaloid content of the seed. The usual (Keller) process[3] when applied to this type of seed was found objectionable as, on account of the high oil content, intractable emulsions were found. The procedure here described was developed especially for seed of high oil content and makes possible a clean separation of the oil and alkaloids.

A mixture of several alkaloids is present in Delphinium seed. These alkaloids are soluble in most of the usual organic solvents, especially chloroform, but not in petroleum ether. Owing to their solubility in the oil of the seed, however, a petroleum ether extract of the seed contains some alkaloids. Ammonia liberates only part of the alkaloids from acid solution, but sodium hydroxide effects complete liberation. Alkaline solutions yield the alkaloids readily to chloroform, but acid solutions are not so rapid in their extraction of the alkaloids from chloroform. Sulphuric acid is the best medium for extracting the alkaloids from an organic solvent, and in this work 5% acid (by weight) was adopted as this strength accomplishes quicker extraction than weaker strengths without harmfully affecting the alkaloids. The alkaloids are partially extracted from chloroform by pure water

PROCEDURE.

Extract 10 Gm. of the well-ground seed (ground as fine as the oily nature will permit) by percolation with low boiling petroleum ether until the oil is apparently all removed. Dry the marc. Extract the petroleum ether solution, concentrated if necessary to about 100 cc., with 5% sulphuric acid until the alkaloids are completely removed. Five extractions of 15 cc. each usually suffice. The last acid extract should give not more than a faint turbidity with Wagner's reagent (iodine in potassium iodide solution), the test being made on approximately 5 cc. of solution. If a precipitate forms, dissolve it by dilute sodium thiosulphate, return to the main solution and continue the extraction.

Filter the petroleum ether extract through paper into a weighed beaker, wash paper and funnel well with the solvent, and evaporate on the steam-bath. If the oil residue is not clear, dissolve it in petroleum ether, filter, wash as before and evaporate again. To insure complete removal of the solvent, which is tenaciously retained by the oil, add a little ethyl alcohol and thoroughly heat on the

* 1001—15th St., Washington, D. C. This work was performed in the U. S. Department of Agriculture.

[1] "The Isolation and Properties of the Alkaloids and Oil of Larkspur Seed (*D. consolida*)," JOUR. A. PH. A., 13 (1924), 696–702.

[2] "Isolation of the Alkaloids and Oil of Stavesacre Seed," JOUR. A. PH. A., 16 (1927), 928–932.

[3] Allen's Commercial Organic Analysis, 4th Edition, Vol. VI, page 179.

steam-bath. Weigh the beaker with contents and from the weight thus obtained calculate the percentage of oil in the seed.

Make the combined acid extracts of the alkaloids distinctly alkaline with 15% sodium hydroxide solution and extract with chloroform, 3 portions of 15 cc. each, or until the aqueous layer gives a negative test for alkaloids. (The test with Wagner's reagent is made in slightly acid solution.) Make the combined chloroform extracts up to exactly 100 cc. and pour over the previously obtained marc in a stoppered flask. After several minutes add 5 cc. of *N* sodium hydroxide solution and shake the flask at frequent intervals for two hours. Filter the contents in a 4-inch Büchner funnel (using a rapid paper), catching the liquid in a suitable vessel such as a calibrated graduate. To avoid evaporation cover the funnel with a watch glass, and do not use suction. Collect as large an aliquot as possible, which will usually be 75 cc.

Extract the chloroform solution repeatedly with 5% sulphuric acid until the last extract fails to give a positive test for alkaloids. Six extractions of 15 cc. each are usually sufficient. Make the aqueous solution distinctly alkaline with sodium hydroxide and extract with chloroform, 3 portions of 15 cc. each, or until the alkaloids are completely removed. For further purification, which is usually necessary and is indicated by the yellow color in the chloroform solution, extract the latter with 5% sulphuric acid as before, again make it alkaline and extract with chloroform. Wash the chloroform solution once with 5–10 cc. of slightly alkaline water and filter through paper into a weighed beaker, washing the separator and funnel well with the solvent. Evaporate the chloroform by means of an air current and moderate heat, add a few cubic centimeters of alcohol, and again evaporate. Warm the beaker a few minutes in the oven (100° C.), cool in a desiccator and weigh. From the weight of the residue calculate the percentage of alkaloids in the seed.

A sample of *D. consolida* and one of *D. staphisagria* were analyzed by this method, with the following results:

D. consolida.		*D. staphisagria.*	
Oil, per cent.	Alkaloids, per cent.	Oil, per cent.	Alkaloids, per cent.
28.3	1.06	34.9	1.30
28.7	1.01	34.7	1.35
28.6	....	35.2	1.22
28.7	....	35.4	1.27

CHEMICAL EXAMINATION OF "PYRIDIUM" AND "MALLOPHENE."

(BRANDS OF PHENYLAZO-ALPHA-ALPHA-DIAMINOPYRIDINE HYDROCHLORIDE.)

BY GEORGE W. COLLINS, PH.C., SC.D.*

Pyridium and Mallophene are proprietary names for two genito-urinary antiseptics recently introduced to the medical profession. The former is marketed by Merck & Co., Inc., Rahway, N. J.; the latter by the Mallinckrodt Chemical Works, St. Louis, Mo. Both Pyridium and Mallophene belong to the azo group

* A contribution from the American Medical Association Chemical Laboratory.